

***Target (protein/gene name):** 6-phosphogluconate dehydrogenase

***NCBI Gene # or RefSeq#:** 5475141R

***Protein ID (NP or XP #) or Wolbachia#:** PVX_117745 (PlasmoDB)

- https://plasmodb.org/plasmo/app/search?q=PVX_117745

***Organism (including strain):** Plasmodium vivax

Etiologic Risk Group (see link below):

***/Disease Information (sort of like the Intro to your Mini Research Write up):**

Plasmodium vivax is a protozoal parasite that is the main source of malaria. P. vivax is one of six species of malaria carriers that infect humans.

Symptoms include fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhoea and abdominal pain. According to DNDi (Drugs for Neglected Diseases initiative), malaria kills about one child every 30 seconds.

Link to TDR Targets page (if present):

http://tdrtargets.org/targets/view?gene_id=268632

Link to Gene Database page (NCBI, EuPath databases -e.g. TryTryp, PlasmoDB, etc - or PATRIC, etc.)

Essentiality of this protein:

Gene/Ortholog: mtu1874 (OG4_10600); **Phenotype:** non-essential; **Source study:** nmpdr

Gene/Ortholog: mtu1142 (OG4_10600); **Phenotype:** essential; **Source study:** nmpdr

Gene/Ortholog: eco1968 (OG4_10600); **Phenotype:** undefined; **Source study:** blattner

Gene/Ortholog: eco1968 (OG4_10600); **Phenotype:** non-essential; **Source study:** gerdes

Gene/Ortholog: eco1968 (OG4_10600); **Phenotype:** non-essential; **Source study:** keio

Gene/Ortholog: eco1968 (OG4_10600); **Phenotype:** non-essential; **Source study:** shigen

Gene/Ortholog: cel16317 (OG4_10600); **Phenotype:** Larval/Adult Lethal/Arrest; **Source study:** neb

Gene/Ortholog: cel16317 (OG4_10600); **Phenotype:** Embryonic Lethal/Arrest; **Source study:** neb

Gene/Ortholog: cel16317 (OG4_10600); **Phenotype:** Embryonic Lethal/Arrest; **Source study:** wormbase

Gene/Ortholog: cel16317 (OG4_10600); **Phenotype:** Larval/Adult Lethal/Arrest; **Source study:** wormbase

Gene/Ortholog: Tb09.211.3180 (OG4_10600); **Phenotype:** significant loss of fitness in bloodstream forms (3 days); **Source study:** alsford

Gene/Ortholog: Tb09.211.3180 (OG4_10600); **Phenotype:** significant loss of fitness in bloodstream forms (6 days); **Source study:** alsford

Gene/Ortholog: Tb09.211.3180 (OG4_10600); **Phenotype:** significant loss of fitness in procyclic forms; **Source study:** alsford

Gene/Ortholog: Tb09.211.3180 (OG4_10600); **Phenotype:** significant loss of fitness in differentiation of procyclic to bloodstream forms; **Source study:** alsford

Is it a monomer or multimer as biological unit? (make prediction at

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html):

Complex of proteins?: No, but the active site may be in chain A or B

Druggable Target (list number or cite evidence from a paper/database showing druggable in another organism): 0.8

***EC#:** 1.1.1.44

Link to BRENDA EC# page:

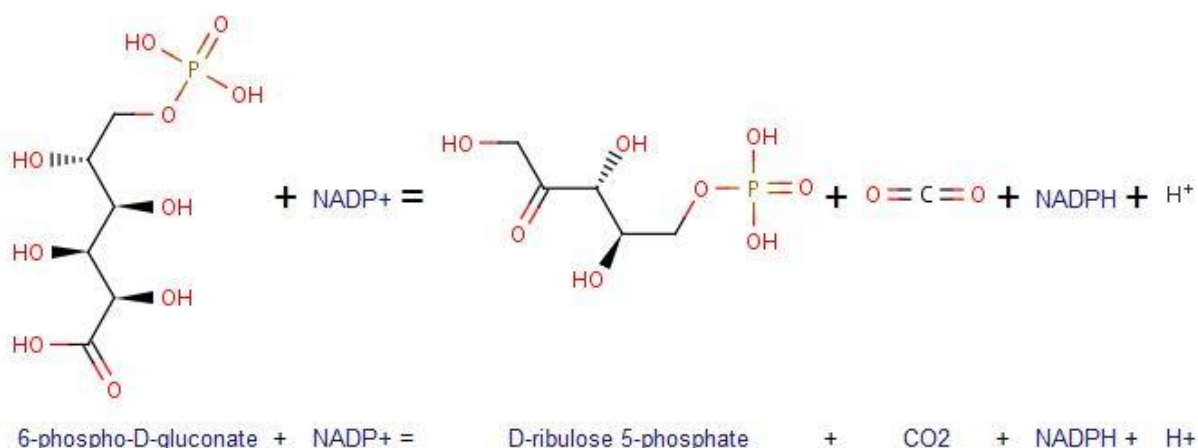
http://www.brenda-enzymes.org/php/result_flat.php4?ecno=1.1.1.44

PDB Site:

<http://www.rcsb.org/pdb/explore/explore.do?structureId=2W8Z> (not of actual organism, but query cover of 98% and ident of 50% homology structure).

-- Show screenshot of BRENDA enzyme mechanism schematic

Reaction catalyzed by phosphogluconate dehydrogenase (NADP+-dependent, decarboxylating) (1.1.1.44)



Enzyme Assay information (spectrophotometric, coupled assay ?, reagents): continuous spectrophotometric rate determination

-- link to Sigma (or other company) page for assay (see Sigma links below)

https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Enzyme_Assay/6phosphoglucdehydrog75.pdf.

-- -or link (or citation) to paper that contains assay information

-- links to assay reagents (substrates) pages.

--- List cost and quantity of substrate reagents, supplier, and catalog #

<http://www.sigmaaldrich.com/catalog/search?interface=All&term=glycylglycine&N=0&mode=match%20partialmax&focus=product&lang=en@ion=US>

Glycylglycine:

Quantity: 132.12g

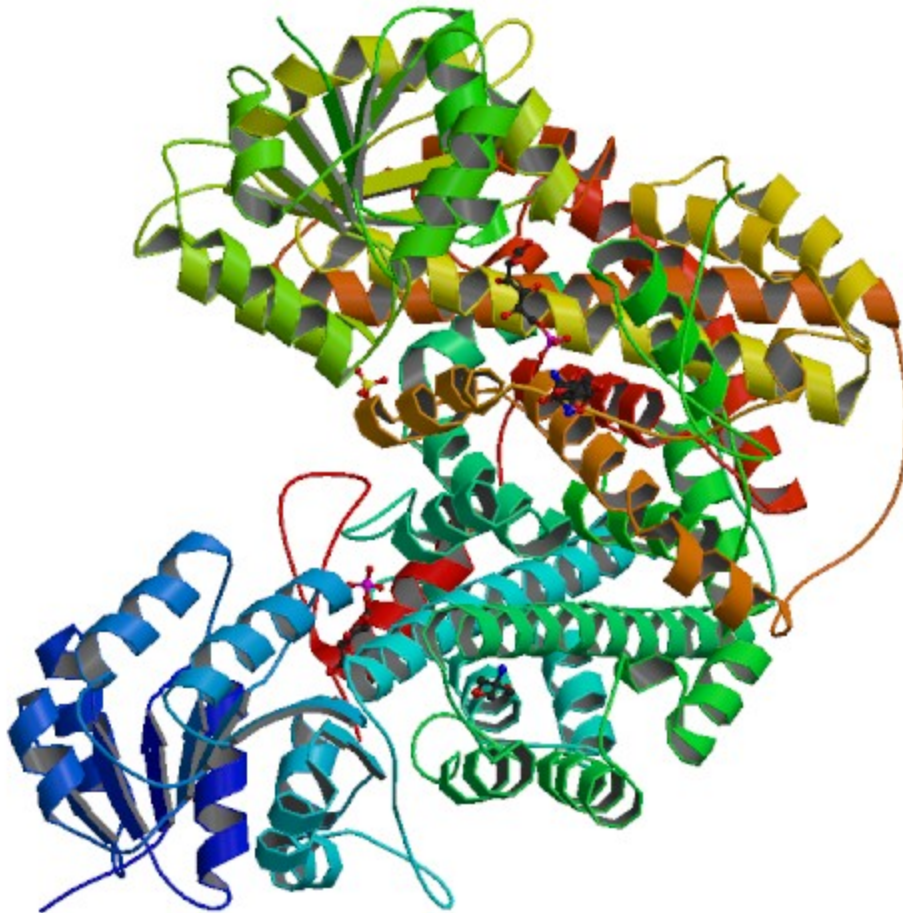
Cost: \$1,170.00

Supplier: PharmaGrade

Catalog: G0674-100G

Structure (PDB or Homology model)

-- PDB # or closest PDB entry if using homology model: Homology model: PDB 2W8Z w/ lig



-- For Homology Model option:

---- Show pairwise alignment of your BLASTP search in NCBI against the PDB

---- Query Coverage:

---- Max % Identities:

---- % Positives

---- Chain used for homology:

Current Inhibitors:

Expression Information (has it been expressed in bacterial cells): expressed in E. coli bacterial cells

Purification Method:

Image of protein (PyMol with features delineated and shown separately):

***Amino Acid Sequence (paste as text only - not as screenshot or as 'code'):**

MTGTCDIGLIGLAVMGQNLSLNIASNGFTIGVYNRTYERTEDTLKKAKEGNLPIQGYETL
EQLINNLLKKPRKIILLIKAGPAVDETIKNILKHFEEDIIIDGGNEWYLNTERITLCEE
HKVEYLAMGVSGGEAGARYGCSFMPGGSKYAYDTIKDILEKCSAKVGTSPCVTYIGPRSS

GNYVKMVHNGIEYGDMQLISESYLLMKNILNYNNEKLSEVFKKWNEGILNSYLIEITYKI
LGKKDELTDNHLVDMILDIAGAKGTGKWTMLEAIERGIPCPTMCAALDARNISAYKQLRV
KADSHFSNDMKVKNKVEGENLINFEDDLLNALYCKIISYTQGLFLLKQVSEEMKWNLNLG
EISRIWRGGCIIRAVFLDRITNAYKKNEKLDLLFLDEDFEQEMKNKLPSLRKVQVATKS
SIPIAFSASLAYFQMVTSQNLPLNLVQAQRDYFGSHTYKRVDRDGDFTIWE

***length of your protein in Amino Acids:** 473 aa

Molecular Weight of your protein in kiloDaltons using the [Expasy ProtParam](http://www.expasy.org/ProtParam) website:
53372.4

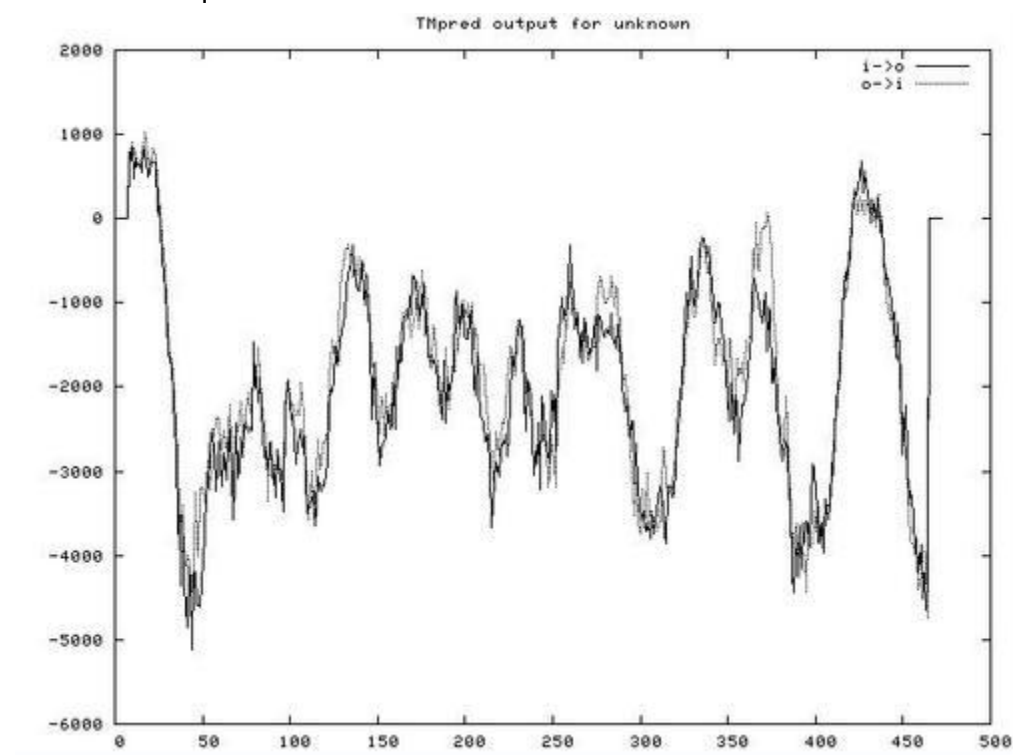
Molar Extinction coefficient of your protein at 280 nm wavelength: 66405 assuming all
pairs of Cys residues form cystines

Literature:

<http://www.sciencedirect.com/science/article/pii/S1047847709002159>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2939770/>

TMpred graph Image (http://www.ch.embnet.org/software/TMPRED_form.html). Input your
amino acid sequence to it.



***CDS Gene Sequence (paste as text only):**

***GC% Content for gene:**

***CDS Gene Sequence (codon optimized) - copy from output of Primer Design Protocol
(paste as text only):** (note this doesn't match the PlasmidDB sequence and shouldn't)

TACTTCCAATCCATGACCGGCACTTGTGATATCGGTCTCATTGGTCTGGCGGTTATGGGTCAGAACCTGTCC
CTGAACATCGCCTCTAACGGTTTTACCATCGGTGTTTACAACCGTACCTACGAGCGTACG

GAAGATACCCTGAAGAAAGCGAAAGAGGGTAACCTGCCGATCCAAGGTATGAAACCCTC
 GAGCAGCTGATCAACAACCTCAAGAAACCGCGTAAAATCATTCTGCTCATCAAAGCGGGT
 CCGGCGGTTGACGAAACCATTAAGAACATCCTGAAGCACTTTGAGGAAGGCGACATCATC
 ATCGACGGTGGTAACGAATGGTACCTCAATACCGAACGTCGTATCACTCTGTGCGAAGAG
 CACAAAGTAGAATACCTGGCGATGGGTGTTTCTGGTGGCGAAGCAGGTGCGCGCTATGGC
 TGTTCCTTTCATGCCGGGTGGCTCCAAATATGCGTATGACACGATCAAAGACATCCTCGAG
 AAGTGCTCTGCGAAAGTTGGCAGGAGCCCTTGCGTAACCTTACATCGGTCCCTCGTAGCTCT
 GGTAACTACGTTAAATGGTTCACAACGGTATCGAATATGGCGATATGCAACTCATCTCC
 GAGTCTTACCTGCTGATGAAAAACATTCTGAACACAATAACGAGAAACTGAGCGAAGTA
 TTCAAAAAATGGAACGAAGGTATCCTCAACTCTTATCTGATCGAAATCACCTATAAGATT
 CTGGGCAAGAAGGACGAACTCACGGACAACCATCTCGTTGACATGATCCTGGACATCGCG
 GGTGCTAAGGGTACGGGTAAATGGACCATGCTGGAAGCGATCGAGCGTGGTATCCCGTGC
 CCGACCATGTGTGCGGCGCTGGACGCCCCGTAATATCTCCGCGTATAAACAGCTGCGCGTT
 AAGGCGGACTCTCATTTTCTAACGACATGAAGGTTAACAAAGTTGAAGGTGAAAATCTC
 ATTAACCTTTGAAGACGACCTCCTGAATGCGCTGTACTGCTGCAAGATCATTTCTTACACC
 CAGGGTCTGTTTCTGCTCAACAGGTTTCTGAAGAGATGAAGTGAATCTCAATCTGGGT
 GAAATCTCTCGTATTTGGCGTGGTGGCTGCATCATCCGCGCTGTCTTCTGGATCGTATT
 ACTAACGCGTACAAGAAGAAATGAAAAGCTCGACCTGCTCTTCTCGACGAGGATTTTCGCG
 CAGGAAATGAAGAACAACTCCCGAGCCTGCGTAAAGTTGTTCAAGTTGCGACCAAATCT
 TCTATCCCGATCCCGCGGTTACGCGCGTCTCTGGCCTACTTTCAGATGGTGACCTCTCAA
 AACCTCCCCTGAACCTGGTGCAGGCGCAACGTGACTACTTCGGCTCTCACACGTATAAG
 CGTGTGACCGTGACGGTGACTTCCATACTATCTGGGAATAACAGTAAAGGTGGATA

***GC% Content for gene (codon optimized): 49.2%**

source: <http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/>

*took CDS gene sequence from primer design tails protocol and input into Oligo Analyzer with 0.4uM Oligo concentration, 50mM Na⁺ concentration, 2mM Mg concentration and 0.3mM dNTP concentration.

Do Not Need this info for Spring (but still copy these lines to your Target page for now)

Primer design results for pNIC-Bsa4 cloning (list sequences of all of your ~40 nt long primers):

(link to DNA Works output text file - that should be saved in your Google Docs folder after you did the primer design protocol)

https://drive.google.com/?pli=1&authuser=0&urp=http://www.google.com/accounts/Logout2?hl%3Den_US%26se#folders/0B_Gl3IMyhDsoSmpQWmV3QTB5OTQ

-- Ask a mentor, Dr. B, or a fellow researcher -how to link a GDocs file if you are not sure how to.

Primer design results for 'tail' primers (this is just 2 sequences):

Forward primer:

5' TACTTCCAATCCATGACCGGCACTTGTGATAT 3'

Reverse primer:

5' TATCCACCTTTACTGTTATTCCCAGATAGTATGGAAGTC 3'

01-Jul-2014

Order No. **10501455**

Ref. No. **123210405**

25 nmole DNA Oligo, 32 bases

Sequence - PhApo-for

5'-TAC TTC CAA TCC ATG ACC GGC ACT TGT GAT AT -3'

Properties	Amount Of Oligo			Shipped To
Tm (30mM NaCl): 62.3 °C	10.2 =	33.8	= 0.33	JOSH BECKHAM
GC Content: 43.8%	OD 260	nMoles	mg	UNIVERSITY OF TEXAS-AUSTIN ICM8 SU
Molecular Weight: 9,734.4				2500 SPEEDWAY
nmol/OD260: 3.3				AUSTIN, TX 78712
up/OD260: 32.4				USA
Ext. Coefficient: 300,500 L/(mole-cm)				5124715022
				Customer No. 171234 PO No. 2013M00003

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -1.84 at 25 °C
Strongest Folding Tm: 39.4 °C

Oligo Base Types	Quantity
DNA Bases	32
Modifications and Services	Quantity
Standard Desalting	1

Disclaimer
See on reverse page notes (I) (R) & (R) for usage, label license, and product warranties

Ref: ID 174449308 Labels - Pool Here



INSTRUCTIONS

*Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

*Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

IDT
INTEGRATED DNA TECHNOLOGIES
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Order No. **10501455**
Ref. No. **123210404**
25 nmole DNA Oligo, 39 bases

01-Jul-2014

Sequence: Polcpa-rev
5'-TAT CCA CCT TTA CTG TTA TTC CCA GAT AGT ATG GAA GTC -3'

Properties	Amount Of Oligo	Shipped To
Tm (50mM NaCl): 80.7 °C	10.5 = 28.1 = 0.33	JOSH BECKHAM
GC Content: 38.3%	OD 260 nmMoles mg	UNIVERSITY OF TEXAS-AUSTIN ICMB SU
Molecular Weight: 11,906.8		2500 SPEEDWAY
nmol/OD260: 2.7		AUSTIN, TX 78712
mg/OD260: 31.8		USA
Ext. Coefficient: 374,700 L/(mole*cm)		\$124715022
		Customer No. 171234 PO No. 2013M00003

Secondary Structure Calculations
Lowest folding free energy (kcal/mole): -1.00 at 25 °C
Strongest Folding Tm: 37.0 °C

Oligo Base Types	Quantity	Disclaimer
DNA Bases	39	See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties
Modifications and Services	Quantity	
Standard Desalting	1	

http://ID1744dP863 Labels - Peel Here

123210404 5'-TAT CCA CCT TTA CTG TTA TTC CCA GAT AGT ATG GAA GTC-3'

Polcpa-rev

10.5 = 28.1 = 0.33

OD 260 nmMoles mg

123210404 5'-TAT CCA CCT TTA CTG TTA TTC CCA GAT AGT ATG GAA GTC-3'

Polcpa-rev

10.5 = 28.1 = 0.33

OD 260 nmMoles mg

INSTRUCTIONS

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Products for Enzyme Activity Assay:
N8035-15VL - Beta NADP+
6PG: P6888 - 10mg